AFRL-OSR-VA-TR-2015-0043

RENEWABLE BIO-SOLAR HYDROGEN PRODUCTION FROM ROBUST OXYGENIC PHOTOTROPHS

Gerard Dismukes
RUTGERS THE STATE UNIVERSITY OF NEW JERSEY

01/22/2015
Final Report

Air Force Research Laboratory
AF Office Of Scientific Research (AFOSR)/ RTE
Arlington, Virginia 22203
Air Force Materiel Command

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
29-01-2015	Final Performance	15-08-2011 to 14-02-2015

4. TITLE AND SUBTITLE
RENEWABLE BIO-SOLAR HYDROGEN PRODUCTION FROM ROBUST OXYGENIC PHOTOTROPHS: THE SECOND GENERATION

5a. CONTRACT NUMBER

5b. GRANT NUMBER
FA9550-11-1-0231

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Gerard Dismukes

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
RUTGERS THE STATE UNIVERSITY OF NEW JERSEY
3 RUTGERS PLZA
NEW BRUNSWICK, NJ 89018559 US

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AF Office of Scientific Research
875 N. Randolph St. Room 3112
Arlington, VA 22203

10. SPONSOR/MONITOR'S ACRONYM(S)
AFOSR

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
A DISTRIBUTION UNLIMITED: PB Public Release

13. SUPPLEMENTARY NOTES

14. ABSTRACT
This project produced advances in two areas: 1) biological hydrogen production, and 2)electrocatalysts. Topic (1) is highlighted by successful genetic engineering of acyanobacterium to redirect the anaerobic catabolism of glycogen, stored from a piorphotosynthetic stage, from the normal glycolytic pathway to an alternative pathway thatproduces significantly more hydrogen. Topic (2) is highlighted by the synthetic of two newelectrocatalysts made from earth-abundant materials that catalyze the oxidation of waterto O2 and the reduction of water to H2, respectively. These were patented and werelicensed commercially where they are in development for applications in commercialelectrolyzers for renewable hydrogen production.

15. SUBJECT TERMS
YIP, renewable, biofuel

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Gerard Dismukes
U	U	U	UU		19b. TELEPHONE NUMBER *(Include area code)* 732-445-1489

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

AFOSR- Annual Report 2014

Principal Investigator Name:	G Charles Dismukes
Primary Contact E-mail:	dismukes@rci.rutgers.edu
Primary Contact Phone Number:	732-445-1489
Grant/Contract Title:	Renewable Bio-Solar Hydrogen Production from Robust Oxygenic Phototrophs: The Second Generation
Grant/Contract Number:	FA9550-11-1-0231
Program Manager:	Patrick Bradshaw
Report Type:	Annual
Reporting Period Start Date:	09/01/2013
Reporting Period End Date:	01/01/2015
Distribution Statement:	
SF298 Form:	
Report Document	

Archival Publications:

2013-2015 AFOSR Funded Publications:

1) Robinson, D.M., Y.B. Go, M. Mui, G. Gardner, Z. Zhang, D. Mastrogiovanni, E. Garfunkel, J. Li, M. Greenblatt and G.C. Dismukes, *Photochemical water oxidation by crystalline polymorphs of manganese oxides: structural requirements for catalysis.* J Am Chem Soc, 2013. **135**(9): p. 3494-501.
2) Xu, Y., L.T. Guerra, Z. Li, M. Ludwig, G.C. Dismukes and D.A. Bryant, *Altered carbohydrate metabolism in glycogen synthase mutants of Synechococcus sp. strain PCC 7002: Cell factories for soluble sugars.* Metab Eng, 2013. **16**: p. 56-67.
3) Guerra, L.T., Y. Xu, N. Bennette, K. McNeely, D.A. Bryant and G.C. Dismukes, *Natural osmolytes are much less effective substrates than glycogen for catabolic energy production in the marine cyanobacterium*

Synechococcus sp. strain PCC 7002. Journal of Biotechnology, 2013. **166**: p. 65– 75.

4) McNeely K., Kumaraswamy G.K., et al. (2014). Metabolic Switching of Central Carbon Metabolism in Response to Nitrate: Application to Autofermentative Hydrogen Production in Cyanobacteria. *Journal of Biotechnology*, 182-183, 83-91.
5) Smith, PF, Kaplan C, Sheats JE, Robinson DM, McCool NS, Mezle N, Dismukes CG. 2014. What determines catalyst functionality in molecular water oxidation? Dependence on ligands and metal nuclearity in cobalt clusters. Inorganic chemistry. 53(4):2113-21.
6) Kumaraswamy, G.K., Tiago Guerra, et al. (2013). Reprogramming the Glycolytic Pathway for Increased Hydrogen Production in Cyanobacteria: Metabolic Engineering of NAD^+-dependent GAPDH. *Energy & Environmental Science*, 6, 3722-3731.

Changes in Research objectives:	none
Change in AFOSR Program Manager, if any:	none
Extensions granted or milestones slipped, if any:	none

100%

1. **Title of Project:** Renewable Bio-Solar Hydrogen Production from Robust Oxygenic Phototrophs: The Second Generation
2. **Grant Number:** FA9550-11-1-0231
3. **Period of this report**: 9/1/13 – 1/1/15
4. **Institution:** The Waksman Institute of Microbiology and the Dept of Chemistry & Chem. Biology, Rutgers The State University of New Jersey, Piscataway, NJ
5. **Telephone & Email:** 732-445-6786; dismukes@rci.rutgers.edu)
6. **Contributing Personnel (20 in total)**:
   - G. Charles Dismukes, PhD, Principal Investigator, Distinguished Professor
   - Gennady Ananyev, PhD, Research Associate Professor
   - Kumaraswamy G. Kenchappa, PhD, Postdoctorate Researcher

- Anagha Krishnan, Busch Scholar graduate fellow, 5th year continuing PhD candidate
- Graeme Gardner, NSF IGERT trainee fellowship, 5th year continuing PhD candidate
- Paul Smith, NSF IGERT trainee fellowship, 5th year continuing PhD candidate
- 6 PhD Rotation Students:
- 10 Undergraduate researchers: Kinan Tadmori, Shannon, Artun Hoscan, Chris Kaplan, Kelly Patraju, Yifan Wang, Gram Townsend, Donald Chawla, Jessica Gonzalez, and Nicholas Mezle.

7. Project Accomplishments & Translational Advances:

- **Trained/Mentored 20 Rutgers Students in Research:** 3 predoctoral trainees in progress, 6 predoctoral rotation students trained, 1 honors thesis (BS) in progress, 10 undergraduate researchers trained.
- **Fundamental Scientific Advances:** 8 publications or submitted manuscripts in peer reviewed journals; 5 of these were done in collaboration with other BioSolarH_2 PIs: Drs. D. Bryant and M. Posewitz. See publication list.
- **Intellectual Property Generated**: A patent disclosure was filed at Rutgers and national patent application was filed on December 31, 2013. NICKEL PHOSPHIDES ELECTROCATALYSTS FOR HYDROGEN EVOLUTION AND OXIDATION REACTIONS. USPTO. PCT/US13/78486.
- **Intellectual Property Generated**: A patent was issued to Rutgers by the USPTO: 13/643,439. SPINEL CATALYSTS FOR WATER AND HYDROCARBON OXIDATION This described the synthesis of a nanoparticulate form of cubic spinel phase of Lithium Cobalt Oxide and demonstration of its function as a highly efficient water oxidation catalyst in electrolyzers.

8. Awards:

- Kumaraswamy G. Kenchappa, Busch postdoc fellowship Waksman Institute

9. Industrial and Public Service Related to AFOSR Sponsored Research

- **Translation of Research to Industry, Licensing Stage:** Licensing of AFOSR funded OER SPINEL CATALYSTS USPTO 13/643,439 to NATCO Corporation.
- **Translation of Research to Industry, Commercial Testing Stage:** Patented OER Spinel catalyst USPTO 13/643,439 has been sent to a commercial electrolyzer company (Proton OnSite, Wallingford, CT) for testing and development of a new form electrolzyer.

10. Public Presentations of AFOSR funded research, 2012-2013

1. Talk: Kumaraswamy G.K, **Metabolic engineering of cyanobacteria for hydrogen production,** *Waksman Annual Retreat,September 9, 2013*

2. Talk: Anagha Krishnan, Metabolic and photosynthetic consequence of mutation of starch biosynthesis in *Chlamydomonas reinhardtii. Waksman Annual Retreat, September 2014*

3. Poster presentation: Title: Metabolic and photosynthetic consequence of

mutation of starch biosynthesis in *Chlamydomonas reinhardtii.*

4. GC Dismukes, invited speaker, **Gordon Research *Conference:* Renewable Energy: Solar Fuels**, Ventura, CA Jan, 19-24 2014.
5. GC Dismukes, 2013 **APRA-E Energy Innovation Summit**,Washington D.C. Feb 25, 2013. Rutgers bioinspired electrocatalysts for solar hydrogen and electrical energy storage.
6. GC Dismukes, **SOFI-Creation of the Solar Fuels Institute**, Telluride, CO, convenor: Dr. M. Wasielewski. Demo Project coPI (2014).
7. Advisory: 2014 DOE-H2A Technoeconomic Review Panel; 2013 DOE-Hydrogen Program;

11. 2013-2014 AFOSR Funded Publications:

1. Robinson, D.M., Y.B. Go, M. Mui, G. Gardner, Z. Zhang, D. Mastrogiovanni, E. Garfunkel, J. Li, M. Greenblatt and G.C. Dismukes, *Photochemical water oxidation by crystalline polymorphs of manganese oxides: structural requirements for catalysis.* J Am Chem Soc, 2013. **135**(9): p. 3494-501.
2. Xu, Y., L.T. Guerra, Z. Li, M. Ludwig, G.C. Dismukes and D.A. Bryant, *Altered carbohydrate metabolism in glycogen synthase mutants of Synechococcus sp. strain PCC 7002: Cell factories for soluble sugars.* Metab Eng, 2013. **16**: p. 56-67.
3. Guerra, L.T., Y. Xu, N. Bennette, K. McNeely, D.A. Bryant and G.C. Dismukes, *Natural osmolytes are much less effective substrates than glycogen for catabolic energy production in the marine cyanobacterium Synechococcus sp. strain PCC 7002.* Journal of Biotechnology, 2013. **166**: p. 65– 75.
4. McNeely K., Kumaraswamy G.K., et al. (2014). Metabolic Switching of Central Carbon Metabolism in Response to Nitrate: Application to Autofermentative Hydrogen Production in Cyanobacteria. *Journal of Biotechnology*, 182-183, 83-91.
5. Smith, PF, Kaplan C, Sheats JE, Robinson DM, McCool NS, Mezle N, Dismukes CG. 2014. What determines catalyst functionality in molecular water oxidation? Dependence on ligands and metal nuclearity in cobalt clusters. Inorganic chemistry. 53(4):2113-21.
6. Kumaraswamy, G.K., Tiago Guerra, et al. (2013). Reprogramming the Glycolytic Pathway for Increased Hydrogen Production in Cyanobacteria: Metabolic Engineering of NAD^+-dependent GAPDH. *Energy & Environmental Science,* 6, 3722-3731.
7. Krishnan, A., Kumaraswamy, G.K., Vinyard, D. J., Gu, H., Ananyev, G., Posewitz, M. P. and Dismukes, G.C. (2015) Metabolic and photosynthetic consequences of blocking starch biosynthesis in the green alga Chlamydomonas reinhardtii sta6 mutant. Submitted to *The Plant Journal.*
8. Xiao Qian, Min Kyung Kim, G. Kenchappa Kumaraswamy, Ananya Agarwal, Desmond S. Lun, and G. Charles Dismukes. Beyond flux balance analysis of

photoautotrophic metabolism: Carbon partitioning into different biopolymers. Submitted.

9. Four more articles are in preparation for publication.

12. Key Scientific Results/Achieved Goals

1. Publication 4 show that the presence of NO_3 in the fermentative media, switch the carbohydrate catabolism through OPP pathway, to meet out the NADPH demand for nitrate reduction. Under nitrate presence, NAD(P)H are largely used for reducing nitrate, rather than H_2. Nitrate presence resulted in 3-fold higher NAD(P)H consumption. Cells largely excrete H_2 and lactate under no nitrate, while CO_2 and nitrite when nitrate present.
2. Publications 6 show that the metabolic pathways for carbohydrate catabolism, especially OPP could yield higher NAD(P)H per glucose catabolised (during autofermentation). This revealed from five cyanobacterial mutant strains studied for their fermentative metabolism during dark/anaerobic conditions. Metabolic engineering of these glycolysis/OPP pathways of carbohydrate catabolism, along with the elimination of competing pathway of lactate production, resulted in enhanced NAD(P)H yields and inturn higher H2 yields. Further we showed that elimination of H_2 in the headspace by either increasing the headspace volume (dilution) or by electrochemical milking shifted the equilibrium of bidirectional hydrogenase towards H2 production rather than consumption.
3. Publication 7 show that during nutrient replete culturing, *sta6* does not redirect metabolism to make more proteins or lipids, and accumulates 20% lower biomass. Above a threshold light intensity, photosynthetic electron transport rate (water→ CO_2) slows in *sta6* due to attenuated rate of NADPH reoxidation, without affecting photosystem I or II (no change in isolated PET). Large accumulations of carbon metabolites were measured that are precursors for biosynthesis of lipids, amino acids and sugars/starch, indicating system-wide consequences of slower NADPH reoxidation. The greatly slower flux through carbon fixation resulted in imbalances in both redox and adenylate energy.
4. Publication 8 presents the experimentally tested most comprehensive genome-scale flux balance model for photoautotrophic growth of the cyanobacterium, *Synechococcus sp.* PCC 7002, which accounts for 768 genes and 769 metabolites. Our model incorporates a variable biomass objective function (*v*BOF) in which stoichiometries of major biomass components vary according to change in light intensity. Modification of the BOF was constrained by measurements of biomass composition under different light conditions. The model provides rigorous agreement to experimentally measured growth rates, inorganic carbon uptake rates and the carbohydrate/protein content as a function of light intensity under photoautotrophic growth. Incorporation of *v*BOF adds greater metabolic flexibility for simulation of more realistic compositional changes in response to light stresses, thus providing more reliable prediction of growth rate and carbon partitioning. Moreover, this model predicts that there exists a gluconeogenesis-OPP hybrid pathway that flux the fixed CO_2 into glycogen.